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REVIEW ARTICLE

Antimicrobial resistance in sexually transmitted infections beyond gonococcal infection

Resistências aos antimicrobianos em infeções sexualmente transmissíveis para além das observadas na infeção gonocócica

Diogo de Sousa[1,] and João Borges Costa[1,2,3]*

[1]Dermatology Department, Hospital de Santa Maria, Centro Hospitalar Universitário de Lisboa Norte; [2]Dermatology Universitary Clinic, Faculdade de Medicina da Universidade de Lisboa; [3]Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa. Lisbon, Portugal

Abstract

Sexually transmitted infections (STIs) show a worldwide growing trend, with a rising incidence in recent years. Although most STIs are not usually lethal, their burden of disease is not negligible resulting in an important health and economic burden worldwide. *Neisseria gonorrhoeae* antimicrobial resistance (AMR) is recognized as a major health concern, with targeted public health response plans worldwide. However, almost all STIs, including syphilis, trichomoniasis, chlamydia, *herpes simplex virus* (HSV) infection, and *Mycoplasma genitalium* infection, have resistances described in the literature, with no structured public health response to assess resistances. This work aims to provide a comprehensive review of AMR within STI beyond gonococcal infection, providing information on prevalence, testing, and treatment recommendations on syphilis, chlamydia, *Trichomonas vaginalis* infection, *M. genitalium* infection, and HSV infection. STIs resistance surveillance must rely on a strong network of case reporting, prevalence analysis, assessment of the etiology of STI syndromes, and monitoring of resistances, to prevent the dissemination and emergence of new resistances.

Keywords: *Chlamydia trachomatis*. Drug resistance. *Mycoplasma genitalium*. Sexually transmitted diseases. Syphilis. Trichomonas infections.

Resumo

As infeções sexualmente transmissíveis demonstram um aumento do número de casos nos últimos anos, com uma incidência crescente. Embora a maioria das infeções sexualmente transmissíveis não seja fatal, as suas consequências mo sociedade não são negligenciáveis, com um importante impacto na saúde e economia das populações. As resistências aos antimicrobianos da bactéria *Neisseria gonorrhoeae* são reconhecidas como um problema global, com programas de resposta em Saúde Pública estabelecidos por todo o mundo. No entanto, a maioria das infeções sexualmente transmissíveis, incluindo a sífilis, clamídia, infeção por *Trichomonas vaginalis*, infeção por *Mycoplasma genitalium* e infeção por *herpes simplex,* têm resistências descritas na literatura, sem programas estruturados de avaliação e monitorização de resistências. O objetivo deste trabalho é fazer uma revisão sobre resistências em infeções sexualmente transmissíveis, para além da gonorreia, com informação sobre prevalência,

Corresponding author:
*Diogo de Sousa
E-mail: 27949@chln.min-saude.pt

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testagem e recomendações de tratamento sobre sífilis, clamídia, infeção por *Trichomonas vaginalis*, infeção por *M. genitalium* e infeção por *herpes simplex.* A vigilância epidemiológica das infeções sexualmente transmissíveis deve ser apoiada por uma rede de notificações de caso, dados sobre prevalência, investigação de síndromes de infeções sexualmente transmissíveis e monitorização de resistências, de modo a prevenir a disseminação e aparecimento de novas estirpes resistentes.

Palavras-chave: *Chlamydia trachomatis*. Resistência a droga. *Mycoplasma genitalium*. Doenças sexualmente transmissíveis. Sífilis. Infecções por trichomonas.

Introduction

Sexually transmitted infections (STIs) show a worldwide growing trend, with an incidence increase of 13% from 2010 to 2019[1]. In 2016, there were an estimated 376.4 million new cases of the four most common curable STIs worldwide: *chlamydia trachomatis* genital infections (127.2 million cases), gonorrhea (86.9 million cases), syphilis (6.3 million cases), and trichomoniasis (156 million cases)[2]. European Union/European Economic Area surveillance data from 2018 reveal that *C. trachomatis* genital infections are the most frequently reported STI in Europe (406,406 cases[3]), followed by gonorrhea (100,673 cases[4]) and syphilis (34,112 cases[5]). These data also indicate that young people (15-24 years old) and men who have sex with men (MSM) are disproportionally affected by bacterial STIs.

Although the most STIs are not usually lethal, their burden of disease is not negligible[1]. The Centers for Disease Control and Prevention (CDC) estimates that one in five people in the United States (US) have an STI. This leads to chlamydia, gonorrhea, and syphilis new infections in 2018 to have totaled nearly $1.1 billion in direct lifetime medical costs alone[6]. Besides the economic burden, these infections have a profound impact on populations health and life quality and may result in fetal and neonatal deaths, pelvic inflammatory disease (PID), chronic pelvic pain, infertility, increased human immunodeficiency virus (HIV) risk, seronegative arthropathy, neurological and cardiovascular diseases and have psychological and social consequences[7].

Antimicrobial resistance (AMR) is a global public health emergency and drug resistance among STIs is no exception[7]. Self-medication, poor antibiotic quality (e.g., substandard or falsified medicines), antibiotic pressure, and international travel play an important role in the growing AMR trend among STIs and its worldwide dissemination[8]. The emergence of AMR in several STIs agents is of major concern, as has significantly compromised treatment effectiveness and control of these infections, especially regarding *Neisseria gonorrhoeae* and *Mycoplasma genitalium*[9]. *N. gonorrhoeae* AMR is recognized as a major health concern, with targeted public health response plans worldwide to identify strategies for enhanced surveillance to detect the emergence of resistances and to mitigate their impact. However, almost all STIs, including syphilis, trichomoniasis, chlamydia, *herpes simplex virus* (HSV) infection, and *M. genitalium* infection, have resistances described in the literature, with no structured public health response to assess resistances.

STI management requires effective, accessible, and inexpensive treatment, supported by adequate prevention, screening, testing, and epidemiological surveillance, including STI resistances. A big setback in recognizing resistances is the difficulty in distinguishing treatment failure from reinfection using nucleic acid amplification tests or cultures, when no standardized test is available for resistances identification. International and European guidelines already focus on AMR, stating recommendations to minimize AMR impact in STI transmission and dissemination. Nonetheless, given the current flexibility on population migration, broader strategies among European countries might play an important role in controlling AMR.

This work aims to provide a comprehensive review of AMR within STI beyond gonorrhea, providing information on prevalence, testing, and treatment recommendations on syphilis, chlamydia, *Trichomonas vaginalis* infection, *M. genitalium* infection, and HSV infection.

Methods

A literature search was conducted through November 13, 2021, using PubMed database with each pathogen reviewed (*C. trachomatis, M. genitalium, Treponema pallidum, T. vaginalis,* and HSV) and the term “resistance.” The authors selected articles that described epidemiology, resistance mechanisms, and treatment recommendations. Additional studies were found using the bibliographies of selected articles and both European and Centers for Disease Control and Prevention (CDC) guidelines were consulted.

Syphilis

Syphilis agent–*T. pallidum pallidum*–is a slender spiral-shaped bacteria usually transmitted through contact with an active lesion of a sexual partner (acquired syphilis) or from an infected pregnant woman to her fetus (congenital syphilis). Syphilis incidence in Europe has shown an overall increase since 2000, which has been mainly due to a significant rise in Western and Central European countries and particularly among MSM[10].

Antibiotics are a key for syphilis infection treatment. The CDC and European guidelines recommend penicillin as first-line treatment for syphilis, with different regimens based on disease stage[11,12]. To this date, there is no report on penicillin-resistant strands of *T. pallidum*, as it continues to be an efficient and widely available treatment. Nonetheless, penicillin allergies and the need for intramuscular administration have required the use of oral antibiotics as alternative treatments. Of these, doxycycline and tetracycline are recommended, and macrolides are not considered a safe alternative in developed countries, as macrolide resistance is of clinical significance[11-14].

The rapid emergence of resistance to azithromycin and clinical failures has been described in several studies, which resulted in azithromycin no longer being recommended as the treatment for syphilis[11,12]. Syphilis treponemes' resistance to macrolide antibiotics has been found to be linked to an *A2058G* or *A2059G* mutation in the *23S rRNA* gene[15]. The *A2058G* mutation encodes resistance to azithromycin, clarithromycin, erythromycin, and roxithromycin but not resistance to spiramycin, and the *A2059G* mutation is presumably associated with resistance to all commonly used macrolides, including spiramycin[14,16,17]. These results indicate that molecular methods to assess *T. pallidum* macrolide resistance must be capable of identifying point mutations at both positions (i.e., 2058 and 2059) in the *23S rRNA* genes for appropriate epidemiological surveillance.

In Europe, genotyped syphilis samples using molecular typing have shown that mutations causing macrolide resistance can range from 66.7% to 94.3%[15,18-21]. The resistance to macrolides seems to be a rising trend, not only in Europe, but a worldwide phenomenon, likely attributed to the pressure generated by the disseminated use of macrolides for other infections (e.g., skin, genital, or oral), including common venereal diseases[22], which also accounts for the low macrolide resistance rates seen in countries with inadequate access to this antibiotic class (e.g., Madagascar and Taiwan)[23,2 4].

Moreover, doxycycline value as an effective oral alternative treatment to syphilis is also confirmed by the absence of genomic resistance to doxycycline in *T. pallidum* strains in Europe[15,21].

Chlamydia

C. trachomatis is an obligate intracellular bacteria transmitted through sexual contact with the penis, vagina, mouth, or anus of an infected partner or spread perinatally from an untreated mother to the baby during childbirth. In 2018, 26 European countries reported 406 406 confirmed chlamydia infections, an increasing number in the past years[3].

The CDC recommends doxycycline as first-line therapy for chlamydia infections and azithromycin or levofloxacin as alternative regimens[11]. As for European guidelines, doxycycline or azithromycin are recommended as a first-line option, followed by erythromycin, levofloxacin, or ofloxacin as second-line alternatives, and josamycin as a third-line choice[25].

Conventionally, evaluating antimicrobial sensitivity of chlamydial strains is technically challenging and time-consuming, as a cell culture is required. Besides the traditional method to demonstrate the ability of *C. trachomatis* to multiply inside the cell in the presence of different concentrations of antibiotics, the resistant strains can also be tested with molecular techniques to identify potential genetic markers of resistance[26]. The occurrence of genetic mutations in *23S rRNA* and tet(M) acquisition is better associated with clinical treatment outcomes of *C. trachomatis* infection than minimum inhibitory concentrations[27]. *C. trachomatis* resistance to macrolides has been described as heterotypic at high infectious loads, a form of phenotypic resistance, not genetically inherited, in which there is the replication of a heterogeneous population of both resistant and susceptible bacteria[13,28].

In clinical setting, > 5% chlamydia failures to the treatment with azithromycin have been reported, with resistances up to 23% in men with non-gonococcal urethritis (NGU) treated with 1 g single-dose azithromycin, although reinfection could not be excluded from the study[28,29]. Macrolide resistance suspicion and doxycycline efficacy in treating chlamydia, particularly in extra urogenital sites, have favored current treatment guidelines into using doxycycline as a first-line approach[29-31].

CDC guidelines recommend all treated for chlamydia to be retested within 3-12 months after the treatment,

although retesting rates are remarkably low[11,32]. European guidelines also advocate for a retest within 3-6 months to patients < 25 years of age. Chlamydia positivity at repeat test has been associated with patients aged < 25 years of age and coinfected with HIV or *Neisseria gonorrhea*[33]. A test of cure (TOC) to detect therapeutic failure, at 4 weeks after completing therapy, should only be sought in pregnancy, if symptoms persist, if therapeutic adherence is in question, or if reinfection is suspected[11,25]. European guidelines also recommend a TOC in extragenital infections, particularly if azithromycin was used[25].

T. vaginalis infection

Trichomoniasis is caused by *T. vaginalis*, a flagellated protozoan parasite of the human urogenital tract and the cause of the most prevalent curable sexually transmitted disease globally[2]. Transmission occurs almost exclusively through sexual contact leading to vaginitis in women and (NGU) in men; however, infection is often asymptomatic, which helps to spread the organism[34]. The prevalence of trichomoniasis is not fully known as it is not a usual mandatory reporting disease; nonetheless, *T. vaginalis* prevalence among European countries has been described to range from 0.3% to 1.4%[35,36].

The recommended treatment for trichomoniasis is based on nitroimidazole compounds, as the lack of a cell wall makes it resistant to classes of antibiotics that target cell wall synthesis, such as penicillin and other beta-lactams. Metronidazole and tinidazole are the first-line recommended treatments[11,37]. The limited therapeutic options available for trichomoniasis treatment push nitroimidazoles resistance to a major emerging health concern.

Metronidazole resistance is difficult to describe due to the lack of uniformity in laboratory testing and resistance definition[38]. *In vitro* metronidazole resistance ranges from 2.7%[39] to 5%[40] in female adolescents, peaking at 9.6% in childbearing women[41]. In the clinical setting, resistances range from 1.2%[42] to 5.7%[41], the latter in a study with metronidazole 2g single-dose treatment. A study in HIV-positive women reported a prevalence of *in vitro* metronidazole resistance of 6%[43] interestingly; other authors have suggested that HIV-positive and HIV-negative women were equally likely to be infected with metronidazole resistant strands[44]. Tinidazole resistance is less well studied, but resistance rates of 1% have been described[45].

T. vaginalis symbiose with *Mycoplasma hominis* seems to play a role in the expression of genes linked to metronidazole *in vitro* resistance; however, the extent to which clinical resistance is affected by this symbiose presence is not yet clear[46]. *T. vaginalis virus* is a dsRNA virus that infects some *T. vaginalis* isolates that might also play a role in *T. vaginalis* resistances, although further investigation is needed[47].

Recurrent *T. vaginalis* infection should prompt an exclusion of reinfection with assessment of the origin. For recurrent or non-responsive to standard therapy trichomoniasis, several alternative regimens are recommended: the first-line treatment is a repeated course of metronidazole; the second-line treatment is a higher dose of metronidazole or tinidazole; and the third-line option is a very high dose of tinidazole, plus intravaginal tinidazole, or paromomycin in women[11,37]. For those failing high-dose nitroimidazole regimens, resistance testing should be performed if available, and the treatment protocol should be guided by the results, even though Europe lacks a reference laboratory to assist on *T. vaginalis* resistance[11,37,48]. If resistance testing is not available, high-dose tinidazole regimens are recommended as there are lower levels of resistance than metronidazole.

M. genitalium infection

M. genitalium is an important cause of urethritis and urogenital syndromes in men and women, associated with persistent or recurrent urethritis and with complications that include PID, sexually acquired reactive arthritis, tubal factor infertility, and epididymitis. In Europe, *M. genitalium* resistance to macrolides shows considerably high rates, with a growing trend in recent years[49], with resistance rates ranging from 5%[50] to 57%[51], in samples taken from males and females attending health services due to urogenital symptoms, partner notification, or high-risk sexual behavior. Fluoroquinolone resistance has been estimated at 5% across European countries[49]. Thus, both macrolide and fluoroquinolone resistance mutations have been described in up to 4% of samples in Sweden[51].

Macrolide resistance in *M. genitalium* is predominantly associated with mutations in *A2059G* and *A2058G*[51]. Mutations within the quinolone resistance -determining region of the *parC* gene have been linked to *in vitro* and *in vivo* moxifloxacin resistance in *M. genitalium*; however, correlation with fluoroquinolone treatment failure is less consistent than that with mutations associated with macrolide resistance[52]. The rising resistance seen in *M. genitalium* is largely attributed to the wide use of macrolides to treat other STIs,

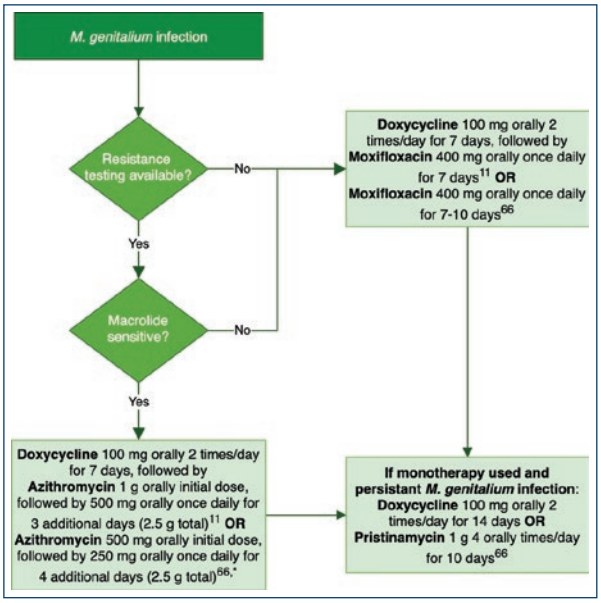

Figure 1. *Mycoplasma genitalium* treatment management following CDC and European guidelines.

particularly as a single dose for *C trachomatis* and *N. gonorrhoeae*[53].

Men with recurrent NGU, women with recurrent cervicitis, and women with PID should be tested for *M. genitalium* using an approved NAAT and the results used to guide therapy[11,54]. Testing should be accompanied by resistance testing by molecular methods, if available[11,54]. If testing is unavailable, *M. genitalium* should be suspected in cases of persistent or recurrent urethritis or cervicitis and considered in PID and treated accordingly[11]. Screening of asymptomatic or extragenital *M. genitalium* infection is not recommended[11].

Either one- or two-stage therapy approaches, ideally using resistance-guided treatment, are recommended for *M. genitalium* treatment (Fig. 1). The CDC recommends for macrolide sensitive *M. genitalium*, doxycycline followed by azithromycin; and for macrolide-resistant or if *M. genitalium* resistance testing is not available, doxycycline followed by moxifloxacin[11]. European guidelines recommend azithromycin or josamycin as first-line options for macrolide sensitive *M. genitalium*, and moxifloxacin if macrolide-resistant strands[54]. For uncomplicated persistent *M. genitalium* symptomatic infection, moxifloxacin is recommended. Recommended third-line treatment for persistent *M. genitalium* symptomatic infection after azithromycin and moxifloxacin is doxycycline or pristinamycin[54].

European guidelines advise for a test of cure at least 3 weeks after the treatment in those who tested positive for *M. genitalium*, as many patients may show few to no symptoms after the treatment, but with persistent carriage and subsequent risk of spreading this infection[54].

HSV infection

HSV-1 and HSV-2 are the two types of HSV that cause genital herpes. The most cases of recurrent genital herpes are caused by HSV-2; however, an increasing proportion of anogenital herpetic infections have been attributed to HSV-1, particularly among young women and MSM[11,55]. Risk of transmission peaks during recurrences or prodrome, even though it can occur in the absence of lesions as a result of subclinical viral shedding[56]. Seroepidemiology studies in European countries have shown HSV-2 seropositivity to range from 4 to 24%, with a higher prevalence among women[57].

Acyclovir resistance should be suspected if a lesion persists or recurs in a patient under antiviral treatment, and the CDC recommends that a viral culture should be obtained for phenotypic sensitivity testing, as molecular testing for acyclovir resistance is not available[11]. Conversely, European guidelines make no recommendation for testing, as testing antiviral susceptibility testing for HSV has limited availability, and state that antiviral treatment should be guided by clinical response[56].

Acyclovir resistant strains have been found in 5-7% isolates from herpes lesions of immunocompromised patients and can it be as high as 36% in hematopoietic stem cell transplant recipients[58-60]. Mutations in viral thymidine kinase or HSV DNA polymerase, or both, are responsible for HSV resistance to acyclovir, though resistance in HSV is predominantly a result of mutations in genes that code for thymidine kinase[61]. Mutations that arise resistance to foscarnet and reduced susceptibility to cidofovir have also been described[62].

Resistance testing of antivirals to HSV can be done by phenotypic and genotypic methods. Phenotypic assays, with the calculation of inhibitory concentrations for the antiviral drug, have the advantage of a clear interpretation of laboratory findings; however, the method is time-consuming, there is a possible selection bias introduced during the growth of heterogeneous viral populations, and experience is required by handling infectious virus[63]. Acyclovir resistance is confirmed if isolates require acyclovir concentrations > 1–3 mg/L for inhibition[56]. Genotypic resistance testing is based on the detection of resistance-related mutations in genes encoding the

thymidine kinase and DNA polymerase. Despite being faster, this method is primarily used to identify modifications already described in the literature, ensuing the identification of unknown mutations, with diverse clinical expression, much harder[63].

All acyclovir-resistant strains are also resistant to valacyclovir, and the majority are resistant to famciclovir; nonetheless, cases are reported of partially resistant strains successfully treated with high-dose intravenous acyclovir and other nucleoside analogs[11,64]. Thymidine kinase defective strains are susceptible to foscarnet and cidofovir, which do not require any activation step to exert their antiviral activity and inhibit viral DNA polymerase[56].

CDC recommends treatment with intravenous foscarnet until clinical resolution is attained for resistant genital HSV. Alternative regimens include intravenous cidofovir, imiquimod 5%, or topical cidofovir gel 1%[11,65,66]. An algorithm for the management of resistant HSV is proposed by Piret and Boivin, with an initial high dose of intravenous acyclovir[63]. New therapeutic options, pritelivir and brincidofovir, an orally bioavailable helicase primase inhibitor and an orally bioavailable lipid ester prodrug of cidofovir, respectively, may help treatment in resistant HSV, but further data are required.

Conclusion

The STI pandemic is on the rise across the globe bringing new therapeutic challenges, as resistant strands to the recommended treatments emerge. *N. gonorrhoeae* is a pathogen with known alarming resistances, and measures have been proposed to mitigate the spreading and emergence of AMR among this infection[7]. Similar actions must be taken regarding other STIs, when considering the rising resistances with common therapeutic options used.

Four core components for STI surveillance are defined by the World Health Organization: case reporting, prevalence assessments, assessment of the etiology of STI syndromes, and monitoring of resistances[7]. Laboratory capacity reinforcement is required for effective resistance surveillance, but also the development and introduction of affordable point-of-care STI diagnostic are a key in providing information to guide therapeutic options and prevent infection spreading and resistances occurrence. To this date, in Portugal, resistance testing on the described STIs is only available for *M. genitalium*, at the National Reference Laboratory for STI.

Robust data on STIs promote more precise and reliable programs, allowing a better allocation of already available resources. Further work focusing on high-risk sexual practices, demography, and STI resistances, as well as strengthening strategic information systems across countries must be put in place to provide policymakers local and regional information to guide health recommendations.

What does the study add?

– Review on STIs antimicrobial resistances beyond gonococcal infection focusing in European epidemiological and pathogenesis data.

– Treatment recommendations according to the latest guidelines issued by European and American health organizations.

– Future recommendations to mitigate the emergence and spread of STIs drug resistance.

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Conflicts of interest

The authors have no conflicts of interest to declare.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

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